

TEST NUMBER: #####
PATIENT NUMBER: #####
GENDER: Female
AGE: 11
DATE OF BIRTH: dd-mm-yyyy

COLLECTED: dd/mm/yyyy
RECEIVED: dd/mm/yyyy
TESTED: dd/mm/yyyy

PRACTITIONER: Nordic Laboratories
ADDRESS:
TEST NAME: Comprehensive Stool Analysis & Parasitology x2 (CSAPx2)
Comprehensive Stool Analysis / Parasitology x2
BACTERIOLOGY CULTURE

Expected/Beneficial flora	Commensal (Imbalanced) flora	Dysbiotic flora
4+ Bacteroides fragilis group	1+ Alpha hemolytic strep	3+ Citrobacter freundii complex
4+ Bifidobacterium spp.	1+ Beta strep, group B	
4+ Escherichia coli	2+ Citrobacter freundii complex, isolate 2	
NG Lactobacillus spp.	2+ Gamma hemolytic strep	
NG Enterococcus spp.	2+ Klebsiella pneumoniae ssp pneumoniae	
	2+ Staphylococcus aureus	
3+ Clostridium spp.		
NG = No Growth		

BACTERIA INFORMATION

Expected /Beneficial bacteria make up a significant portion of the total microflora in a healthy & balanced GI tract. These beneficial bacteria have many health-protecting effects in the GI tract including manufacturing vitamins, fermenting fibers, digesting proteins and carbohydrates, and propagating anti-tumor and anti-inflammatory factors.

Clostridia are prevalent flora in a healthy intestine. Clostridium spp. should be considered in the context of balance with other expected/beneficial flora. Absence of clostridia or over abundance relative to other expected/beneficial flora indicates bacterial imbalance. If *C. difficile* associated disease is suspected, a Comprehensive Clostridium culture or toxigenic *C. difficile* DNA test is recommended.

Commensal (Imbalanced) bacteria are usually neither pathogenic nor beneficial to the host GI tract. Imbalances can occur when there are insufficient levels of beneficial bacteria and increased levels of commensal bacteria. Certain commensal bacteria are reported as dysbiotic at higher levels.

Dysbiotic bacteria consist of known pathogenic bacteria and those that have the potential to cause disease in the GI tract. They can be present due to a number of factors including: consumption of contaminated water or food, exposure to chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress levels.

YEAST CULTURE

Normal flora	Dysbiotic flora
1+ Saccharomyces cerevisiae/bouardii	

MICROSCOPIC YEAST

Result:	Expected:
Rare	None - Rare

Yeast in stool is expected at a level of none-rare. A microscopic finding of yeast in stool of few, moderate, or many may be helpful in identifying potential yeast overgrowth, or non-viable or dietary yeast.

YEAST INFORMATION

Yeast may normally be present in small quantities in the skin, mouth, and intestine. When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast are not uniformly dispersed throughout the stool and this may lead to undetectable or low levels of yeast identified by microscopy, despite culture and identified yeast species. Conversely, microscopic examination may reveal a significant amount of yeast present but no viable yeast cultured. Yeast may not always survive transit through the intestines. Nonviable diet-derived yeast may also be detected microscopically. Consideration of clinical intervention for yeast detected microscopically should be made in the context of other findings and presentation of symptoms.

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*** Aeromonas, Campylobacter, Plesiomonas, Salmonella, Shigella, Vibrio, Yersinia, & Edwardsiella tarda have been specifically tested for and found absent unless reported.**



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DIGESTION / ABSORPTION				<p>Elastase findings can be used for the diagnosis or the exclusion of exocrine pancreatic insufficiency. Correlations between low levels and chronic pancreatitis and cancer have been reported. Fat Stain: Microscopic determination of fecal fat using Sudan IV staining is a qualitative procedure utilized to assess fat absorption and to detect steatorrhea. Muscle fibers in the stool are an indicator of incomplete digestion. Bloating, flatulence, feelings of "fullness" may be associated with increase in muscle fibers. Vegetable fibers in the stool may be indicative of inadequate chewing, or eating "on the run". Carbohydrates: The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.</p>
	Within	Outside	Reference Range	
Elastase	411		> 200 µg/mL	
Fat Stain	None		None - Mod	
Muscle fibers	None		None - Rare	
Vegetable fibers	Rare		None - Few	
Carbohydrates	Neg		Neg	

INFLAMMATION				<p>Lactoferrin and Calprotectin are reliable markers for differentiating organic inflammation (IBD) from functional symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse. Lysozyme* is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients. White Blood Cells (WBC) and Mucus in the stool can occur with bacterial and parasitic infections, with mucosal irritation, and inflammatory bowel diseases such as Crohn's disease or ulcerative colitis.</p>
	Within	Outside	Reference Range	
Lactoferrin	< 0.5		< 7.3 µg/mL	
Calprotectin*	< 10		<= 50 µg/g	
Lysozyme*	150		<= 600 ng/mL	
White Blood Cells	None		None - Rare	
Mucus	Neg		Neg	

IMMUNOLOGY				<p>Secretory IgA* (sIgA) is secreted by mucosal tissue and represents the first line of defense of the GI mucosa and is central to the normal function of the GI tract as an immune barrier. Elevated levels of sIgA have been associated with an upregulated immune response.</p>
	Within	Outside	Reference Range	
Secretory IgA*		31.5	51 - 204 mg/dL	

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*For Research Use Only. Not for use in diagnostic procedures.

Methodology: Elisa, Microscopy, Colormetric, Gas Chromatography, ph Electrode

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SHORT CHAIN FATTY ACIDS

	Within	Outside	Reference Range	
% Acetate	71		40 - 75 %	<p>Short chain fatty acids (SCFAs): SCFAs are the end product of the bacterial fermentation process of dietary fiber by beneficial flora in the gut and play an important role in the health of the GI as well as protecting against intestinal dysbiosis. Lactobacilli and bifidobacteria produce large amounts of short chain fatty acids, which decrease the pH of the intestines and therefore make the environment unsuitable for pathogens, including bacteria and yeast. Studies have shown that SCFAs have numerous implications in maintaining gut physiology. SCFAs decrease inflammation, stimulate healing, and contribute to normal cell metabolism and differentiation. Levels of Butyrate and Total SCFA in mg/mL are important for assessing overall SCFA production, and are reflective of beneficial flora levels and/or adequate fiber intake.</p>
% Propionate	17		9 - 29 %	
% Butyrate	11		9 - 37 %	
% Valerate	1.2		0.5 - 7 %	
Butyrate	0.96		0.8 - 4.8 mg/mL	
Total SCFA's	8.6		4 - 18 mg/mL	

INTESTINAL HEALTH MARKERS

	Within	Outside	Reference Range	
Red Blood Cells	None		None - Rare	<p>Red Blood Cells (RBC) in the stool may be associated with a parasitic or bacterial infection, or an inflammatory bowel condition such as ulcerative colitis. Colorectal cancer, anal fistulas, and hemorrhoids should also be ruled out.</p> <p>pH: Fecal pH is largely dependent on the fermentation of fiber by the beneficial flora of the gut.</p> <p>Occult blood: A positive occult blood indicates the presence of free hemoglobin found in the stool, which is released when red blood cells are lysed.</p>
pH	6.6		6 - 7.8	
Occult Blood	Neg		Neg	

MACROSCOPIC APPEARANCE

	Appearance	Expected	
Color	Brown	Brown	<p>Color: Stool is normally brown because of pigments formed by bacteria acting on bile introduced into the digestive system from the liver. While certain conditions can cause changes in stool color, many changes are harmless and are caused by pigments in foods or dietary supplements. Consistency: Stool normally contains about 75% water and ideally should be formed and soft. Stool consistency can vary based upon transit time and water absorption.</p>
Consistency	Solid/Dry	Formed/Soft	

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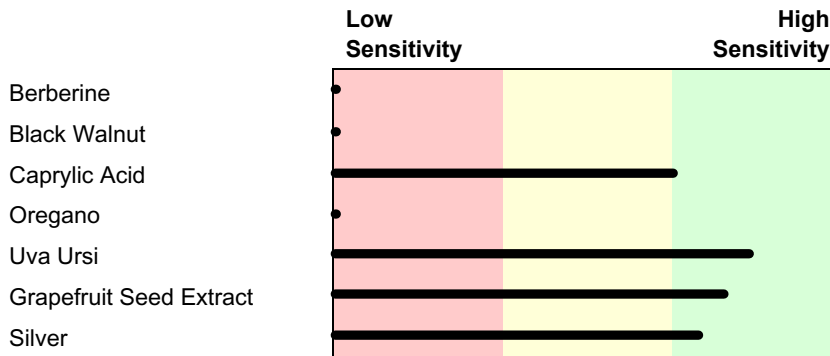
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Bacterial Susceptibilities: Citrobacter freundii complex

NATURAL ANTIBACTERIALS



Natural antibacterial agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based upon the diameter of the zone of inhibition surrounding the disk. Data based on over 5000 individual observations were used to relate the zone size to the activity level of the agent. A scale of relative sensitivity is defined for the natural agents tested.

PRESCRIPTIVE AGENTS

	Resistant	Intermediate	Susceptible
Amoxicillin-Clavulanic Acid	R		
Ampicillin	R		
Cefazolin	R		
Ceftazidime			S
Ciprofloxacin			S
Trimeth-sulfa			S

Susceptible results imply that an infection due to the bacteria may be appropriately treated when the recommended dosage of the tested antimicrobial agent is used.
Intermediate results imply that response rates may be lower than for susceptible bacteria when the tested antimicrobial agent is used.
Resistant results imply that the bacteria will not be inhibited by normal dosage levels of the tested antimicrobial agent.

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**Natural antibacterial agent susceptibility testing is intended for research use only.
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Yeast Susceptibilities: Saccharomyces cerevisiae/boulardii

NATURAL ANTIFUNGALS		
	Low Sensitivity	High Sensitivity
Berberine		
Caprylic Acid		
Uva Ursi		
Plant Tannins		
Oregano		
Undecylenic Acid		
Grapefruit Seed Extract		

Natural antifungal agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based upon the diameter of the zone of inhibition surrounding the disk. Data based on over 5000 individual observations were used to relate the zone size to the activity level of the agent. A scale of relative sensitivity is defined for the natural agents tested.

NON-ABSORBED ANTIFUNGALS		
	Low Sensitivity	High Sensitivity
Nystatin		

Non-absorbed antifungals may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed using standardized commercially prepared disks impregnated with Nystatin. Relative sensitivity is reported based upon the diameter of the zone of inhibition surrounding the disk.

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INTRODUCTION

This analysis of the stool specimen provides fundamental information about the overall gastrointestinal health of the patient. When abnormal microflora or significant aberrations in intestinal health markers are detected, specific interpretive paragraphs are presented. If no significant abnormalities are found, interpretive paragraphs are not presented.

Clostridium spp

Clostridia are expected inhabitants of the human intestine. Although most clostridia in the intestine are not virulent, certain species have been associated with disease. Clostridium perfringens is a major cause of food poisoning and is also one cause of antibiotic-associated diarrhea. Clostridium difficile is a causative agent in antibiotic-associated diarrhea and pseudomembranous colitis. Other species reported to be prevalent in high amounts in patients with Autistic Spectrum Disorder include Clostridium histolyticum group, Clostridium cluster I, Clostridium bolteae, and Clostridium tetani.

If these disease associations are a concern further testing may be necessary.

Washington W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods, G. Koneman’s Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Lippincott Williams and Wilkins; 2006. pg 931-939

Song Y, Liu C, Finegold SM. Real-Time PCR Quantitation of Clostridia in Feces of Autistic Children. Applied and Environmental Microbiology. Nov. 2004, 6459-6465.

Parracho H, Bingham MO, Gibson GR, McCartney AL. Differences Between the Gut Microflora of Children with Autistic Spectrum Disorders and That of Healthy Children. Journal of Medical Microbiology. 2005;54, 987-991.

Imbalanced flora

Imbalanced flora are those bacteria that reside in the host gastrointestinal tract and neither injure nor benefit the host. Certain dysbiotic bacteria may appear under the imbalances category if found at low levels because they are not likely pathogenic at the levels detected. When imbalanced flora appear, it is not uncommon to find inadequate levels of one or more of the beneficial bacteria and/or a fecal pH which is more towards the alkaline end of the reference range (6 - 7.8). It is also not uncommon to find hemolytic or mucoid E. coli with a concomitant deficiency of beneficial E. coli and alkaline pH, secondary to a mutation of beneficial E. coli in alkaline conditions (DDI observations). Treatment with antimicrobial agents is unnecessary unless bacteria appear under the dysbiotic category.

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Mackowiak PA. The normal microbial flora. N Engl J Med. 1982;307(2):83-93.

Dysbiotic Flora

In a healthy balanced state of intestinal flora, the beneficial bacteria make up a significant proportion of the total microflora. However, in many individuals there is an imbalance or deficiency of beneficial flora and an overgrowth of non-beneficial (imbalance) or even pathogenic microorganisms (dysbiosis). This can be due to a number of factors including: consumption of contaminated water or food; daily exposure of chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress levels.

A number of toxic substances can be produced by the dysbiotic bacteria including amines, ammonia, hydrogen sulfide, phenols, and secondary bile acids which may cause inflammation or damage to the brush border of the intestinal lining. If left unchecked, long-term damage to the intestinal lining may result in leaky gut syndrome, allergies, autoimmune disease (e.g. rheumatoid arthritis), irritable bowel syndrome, fatigue, chronic headaches, and sensitivities to a variety of foods. In addition, pathogenic bacteria can cause acute symptoms such as abdominal pain, nausea, diarrhea, vomiting, and fever in cases of food poisoning.

Bacterial sensitivities to a variety of prescriptive and natural agents have been provided for the pathogenic bacteria that were cultured from this patient’s specimen. This provides the practitioner with useful information to help plan an appropriate treatment regimen. Supplementation with probiotics or consumption of foods (yogurt, kefir, miso, tempeh, tamari sauce) containing strains of lactobacilli, bifidobacteria, and enterococci can help restore healthy flora levels. Polyphenols in green and ginseng tea have been found to increase the numbers of beneficial bacteria. Hypochlorhydria may also predispose an individual to bacterial overgrowth, particularly in the small intestine. Nutritional anti-inflammatories can aid in reversing irritation to the GI lining. These include quercetin, vitamin C, curcumin, gamma-linoleic acid, omega-3 fatty acids (EPA, DHA), and aloe vera. Other nutrients such as zinc, beta-carotene, pantothenic acid, and L-glutamine provide support for regeneration of the GI mucosa. A comprehensive program may be helpful in individuals in whom a dysbiotic condition has caused extensive GI damage.

Lispi E. Digestive Wellness. New Canaan,CT: Keats Publishing;1996.

Mitsuoka T. Intestinal Flora and Aging. Nutr Rev 1992;50(12):438-446.

Weisburger JH. Tea and Health: The Underlying Mechanisms. Proc Soc Exp Biol Med 1999;220(4):271-275.4.

Pereira SP, Gainsborough N, Dowling RH. Drug-induced Hypochlorhydria Causes High Duodenal Bacterial Counts in the Elderly. Ailment Pharmacol Ther 1998;12(1)99-104.

Murray MT. Stomach Ailments and Digestive Disturbances. Rocklin, CA: Prima Publishing; 1997.

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Citrobacter species

Citrobacter species, a gram-negative bacterium and member of the Enterobacteriaceae family, is considered dysbiotic at 3+ or greater.

Citrobacter freundii complex, including Citrobacter freundii, Citrobacter braakii, Citrobacter gullenii, Citrobacter murlinae, Citrobacter rodentium, Citrobacter werkmanii, Citrobacter oungae, and less commonly, Citrobacter koseri and Citrobacter farmeri, can cause diarrheal disease. Symptoms due to Citrobacter freundii complex seem to be a result of the elaboration of an E. coli-like heat-stable enterotoxin and hydrogen sulfide. Citrobacter freundii complex has been implicated as a cause of gastrointestinal infection and inflammation, acute dysentery, and dyspepsia. Acute symptoms can include profuse, watery diarrhea which is often unaccompanied by abdominal pain, fecal blood, or white blood cells.

Citrobacter species thrive on Fructooligosaccharides (FOS), a common ingredient in artificial or alternative sweetener.

Antibiotics may be indicated if symptoms are prolonged. Refer to the bacterial sensitivities to identify the most appropriate agent.

Guarino, A, et al. Production of Escherichia coli Sta-Like Heat-Stable Enterotoxin by Citrobacter freundii Isolated from Humans. Journal of Clinical Microbiology. January 1987;110-114.

Washington W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods, G. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Lippincott Williams and Wilkins; 2006. pg 686-689.

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC. Manual of Clinical Microbiology, 8th edition. Washington, DC: ASM Press; 2003. pg 701-704.

Cultured Yeast

Yeast, such as Candida are normally present in the GI tract in very small amounts. Many species of yeast exist and are commensal; however, they are always poised to create opportunistic infections and have detrimental effects throughout the body. Factors that contribute to a proliferation of yeast include frequent use of wide-spread antibiotics/low levels of beneficial flora, oral contraceptives, pregnancy, cortisone and other immunosuppressant drugs, weak immune system/low levels of sIgA, high-sugar diet, and high stress levels.

When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast grows in colonies and is typically not uniformly dispersed throughout the stool. This may lead to undetectable or low levels of yeast identified by microscopy, despite a cultured amount of yeast. Conversely, microscopic examination may reveal a significant amount of yeast present, but no yeast cultured. Yeast does not always survive transit through the intestines rendering it unviable for culturing. Therefore, both microscopic examination and culture are helpful in determining if abnormally high levels of yeast are present.

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Secretory IgA (sIgA)

Immunological activity in the gastrointestinal tract can be assessed using secretory immunoglobulin A (sIgA) in a formed stool sample. However, sIgA may be artefactually low due to fluid dilution effects in a watery or loose/watery stool sample. Secretory IgA is the predominant antibody, or immune protein the body manufactures and releases in external secretions such as saliva, tears, and milk [1]. It is also transported through the epithelial cells that line the intestines out into the lumen. Secretory IgA represents the first line of defense of the GI mucosa and is central to the normal function of the GI tract as an immune barrier [1]. As the principal immunoglobulin isotype present in mucosal secretions, sIgA plays an important role in controlling intestinal milieu which is constantly presented with potentially harmful antigens such as pathogenic bacteria, parasites, yeast, viruses, abnormal cell antigens, and allergenic proteins [1]. Secretory IgA antibodies exert their function by binding to antigenic epitopes on the invading microorganism, limiting their mobility and adhesion to the epithelium of the mucus membrane [2]. This prevents the antigens from reaching systemic circulation and allowing them to be excreted directly in the feces.

Mental and physical stress as well as inadequate nutrition have been associated with low fecal sIgA concentrations. This includes dietary restrictions, excessive alcohol intake, body mass loss, negative moods, and anxiety [3]. One study found depressed levels of sIgA in malnourished children, particularly protein malnourishment, that responded well to nutritional rehabilitation with a significant increase in sIgA [4]. This may be because the synthesis and expression of sIgA requires adequate intake of the amino acid L-glutamine [3]. Animal studies have demonstrated that a glutamine-restricted diet can result in a 50% decrease in sIgA levels [5]. An increase of dietary L-glutamine can restore GI immune function by protection of cells that synthesize sIgA [6]. *Saccharomyces boulardii* is a nonpathogenic yeast that has been used for the treatment of acute infectious enteritis and antibiotic-associated diarrhea [7]. Significantly elevated levels of sIgA and subsequent enhanced host immune response have been found following *S. boulardii* administration in mice and rats [8,9].

References:

1. Crago SS, Tomasi TB. Mucosal Antibodies, Food Allergy and Intolerance. Bailliere Tindall/W.B. Saunders 1987;167-89.
2. Roberts JA. Factors predisposing to urinary tract infections in children. *Ped Neph* 1996;10:517-522.
3. Carins J, Booth C. Salivary immunoglobulin-A as a marker of stress during strenuous physical training. *Aviat Space Environ Med* 2002;73(12)1203-7.
4. Teodosio MR, Oliveira ECM. Urinary secretory IgA after nutritional rehabilitation. *Braz J Med Biolog Res* 1999;32:421-426
5. Alverdy J. Effects of glutamine-supplemented diets on immunology of the gut. *J Parent Enteral Nutr* 1990;14(4):1095-1135.
6. Burke DJ, et al. Glutamine-supplemented total parenteral nutrition improves gut function. *Arch Surg* 1989;24:2396-2399.
7. Alverdy JA. The effect of total parenteral nutrition on gut lamina propria cells. *J Parent. Enteral Nutr* 1990;14(suppl).
8. Qamar A, Aboudola S, Warny M, et al. *Saccharomyces boulardii* stimulates intestinal immunoglobulin A immune response to clostridium difficile toxin A in

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- mice. Infect Immun 2001;69(4):2762-5.
9. Buts JP, Bernasconi P, Vaerman JP, et al. Stimulation of secretory IgA and secretory component of immunoglobulins in small intestine of rats treated with *Saccharomyces boulardii*. Dig Dis Sci 1990;35(2):251-6.

Beneficial Flora

One or more of the expected or beneficial bacteria are low in this specimen. Normally abundant include lactobacilli, bifidobacteria, clostridia, *Bacteroides fragilis* group, enterococci, and some strains of *Escherichia coli*. The beneficial flora have many health-protecting effects in the gut, and as a consequence, are crucial to the health of the whole organism. Some of the roles of the beneficial flora include digestion of proteins and carbohydrates, manufacture of vitamins and essential fatty acids, increase in the number of immune system cells, break down of bacterial toxins and the conversion of flavinoids into anti-tumor and anti-inflammatory factors. Lactobacilli, bifidobacteria, clostridia, and enterococci secrete lactic acid as well as other acids including acetate, propionate, butyrate, and valerate. This secretion causes a subsequent decrease in intestinal pH, which is crucial in preventing an enteric proliferation of microbial pathogens, including bacteria and yeast. Many GI pathogens thrive in alkaline environments. Lactobacilli also secrete the antifungal and antimicrobial agents lactocidin, lactobacillin, acidolin, and hydrogen peroxide. The beneficial flora of the GI have thus been found useful in the inhibition of microbial pathogens, prevention and treatment of antibiotic associated diarrhea, prevention of traveler's diarrhea, enhancement of immune function, and inhibition of the proliferation of yeast.

In a healthy balanced state of intestinal flora, the beneficial bacteria make up a significant proportion of the total microflora. Healthy levels of each of the beneficial bacteria are indicated by either a 2+, 3+ or 4+ (0 to 4 scale). However, in some individuals there is an imbalance or deficiency of beneficial flora and an overgrowth of non-beneficial (imbalance) or even pathogenic microorganisms (dysbiosis). This can be due to a number of factors including: consumption of contaminated water or food; daily exposure of chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress levels.

A number of toxic substances can be produced by the dysbiotic bacteria including amines, ammonia, hydrogen sulfide, phenols, and secondary bile acids which may cause inflammation or damage to the brush border of the intestinal lining. If left unchecked, long-term damage to the intestinal lining may result in leaky gut syndrome, fatigue, chronic headaches, and sensitivities to a variety of foods. In addition, pathogenic bacteria can cause acute symptoms such as abdominal pain, nausea, diarrhea, vomiting and fever in cases of food poisoning.

Antibacterial and antifungal susceptibility testing to a variety of prescriptive and natural agents may be provided for the pathogenic organisms that are cultured from this patient's specimen. This testing is intended to provide the practitioner with useful information to help plan an appropriate treatment regimen. A comprehensive program may be helpful in individuals in whom a dysbiotic condition has caused extensive GI damage.

Note: Not all genera or species can be tested for susceptibility in the laboratory due to their



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specific growth requirements. In addition, the Centers for Disease Control and prevention recommend not testing certain organisms such as those associated with food poisoning. If a practitioner has specific questions, please contact customer service.

Percival M. Intestinal Health. Clin Nutr In. 1997;5(5):1-6.

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Nordic Laboratories Aps

Nygade 6, 3.sal • 1164 Copenhagen K • Denmark
Tel: +45 33 75 10 00

UK Office:

11 Old Factory Buildings • Stonegate • E. Sussex TN5 7DU • UK
Tel: +44 (0)1580 201 687